

OCCURRENCE OF ANTIBIOTIC RESISTANT ENTEROCOCCI ON SKIN OF TEATS AND TEAT CUPS OF MILKING MACHINE

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ABSTRACT

The aim of this study was to investigate the occurrence of enterococci in conditions of farmhouse production and antibiotic resistance of 49 strains isolated from skin of teats and 50 strains isolated from teat cups of milking machine. The samples for enumeration and isolation of enterococci were cultured on selective diagnostic Slanetz-Bartley agar. The isolates were phenotypically identified at the species level with EN-COCCUS test. Susceptibilities of isolated enterococci to antibiotic (vancomycin 30 µg/disc, ampicillin 10 µg/disc, erythromycin 15 µg/disc, gentamicin 120 µg/disc, teicoplanin 30 µg/disc and tetracycline 30 µg/disc) were tested using the disc diffusion method. The counts of enterococci from teats reached the average value $2.77 \log_{10} \text{ cfu.ml}^{-1}$ and from teat cups of milking machine average value $2.85 \log_{10} \text{ cfu.ml}^{-1}$. The dominant species isolated from teats (41%) and also from teat cups (36%) were representatives of *E. group III* - *E. durans*, *E. hirae*, *E. faecalis asaccharolytic var.* The isolates of enterococci obtained from teats showed resistance to tetracycline (4%), erythromycin (4%) and intermediary resistance to tetracycline (8%), erythromycin (25%) and to vancomycin (8%). The isolates of enterococci obtained from teat cups showed resistance to tetracycline (2%) and intermediary resistance to erythromycin (20%) and to vancomycin (6%). No resistance to the antibiotic teicoplanin, ampicillin and gentamicin was found. The most of the isolates of enterococci were sensitive to the tested antibiotics.

Keywords: enterococci; antibiotic resistance; teat; teat cup

INTRODUCTION

The bacteria of the genus *Enterococcus*, also known as “enterococci”, are part of the environmental, food and clinical microbiology. Depending on the strains, they are considered as indicators of faecal contamination, spoilage, or potentially pathogenic organisms, but they have also positive effects (production of aromatic compounds, bacteriocins, etc.).

Enterococci are Gram-positive, facultative anaerobic bacteria. The majority of *Enterococcus* species is capable of growth at 10 and 45 °C, in 6.5% sodium chloride, at pH 9.6 and can survive heating at 60 °C for 30 min (Hardie, Whiley, 1997).

These microorganisms are frequently associated with many foods from animal (dairy and meat products) and vegetable origins (Franz et al., 2003; Giraffa, 2003; Todorov, Dicks, 2005; Dal Bello et al., 2010; Fabianová et al., 2010). The reason for the prevalence of enterococci in dairy products has long been considered as a result of unhygienic conditions during the production and processing of milk. However, their presence in foods has often been shown to be unrelated with direct faecal contamination (Giraffa, 2003; Elmosmelany et al., 2009). In general, microbial contamination of raw milk occurs from three main sources: from within the udder, from the exterior of the udder, and from the surface of milk handling and storage equipment (Bramley, McKinnon, 1990; McKinnon et al., 1990; Jayarao, Wolfgang, 2003; Lavová et al., 2011; Čanigová et al., 2012).

Enterococcus spp. are used as indicator bacteria for the development of antibiotic resistance (Danish Integrated Antimicrobial Resistance Monitoring and Research Programme - DANMAP, 2003), and provide accurate information on previous antibiotic treatment of the animals (Centers for Disease Control and Prevention - CDC, 2002). Enterococci are known to acquire antibiotic resistance with relative ease and to be able to spread these resistance genes to other species (Kühn et al., 2000). However, there is little information about the prevalence and epidemiology of antibiotic resistance of enterococci outside of the hospital setting, including dairy farms (Giraffa, 2003; Nam et al., 2010).

With this in mind, the objectives of the present study were: (1) enumeration and isolation of enterococci from skin of teats and from teat cups of milking machine; (2) determination of the species distribution and the antibiotic resistance of the enterococci isolated from teats and teat cups.

MATERIAL AND METHODOLOGY

Enumeration and isolation of enterococci

Swabs from teat skin and teat cups were taken by sterile tampons moistened in sterile saline solution in two different dairy farms with various hygienic conditions. Swab of teat was performed from teat surface after its cleaning before milking and swab of teat cup before installing milking machine to udder. Decimal dilutions of the samples were plated on Slanetz-Bartley selective agar

(Biokar Diagnostic, France) and incubated at 37 ± 1 °C for 48 ± 2 h. The counts of colonies with enterococcal morphology were enumerated after incubation. The typical colonies of enterococci were randomly selected from plates for isolation, from skin of teats (n=58) and from teat cups of milking machine (n=59).

Phenotypic identification

The genus *Enterococcus* was confirmed microscopically after Gram staining, using the catalase test with 3% H₂O₂, pyrrolidonylamidase test – PYRA test (Lachema, Czech Republic), bile-esculin test on bile, esculin and azid agar (Biokar Diagnostic, France) in 49 isolates from teats and 50 isolates from teat cups. The isolates of enterococci were phenotypically identified at the species level with EN-COCCUS test (Lachema, Czech Republic).

Determination of antibiotic resistance

The strains of enterococci were tested for antibiotic resistance on Mueller-Hinton agar (HiMedia, India) by the standard disc diffusion method (CLSI, 2011). The discs of therapeutically used antibiotics vancomycin (30 µg/disc), ampicillin (10 µg/disc), erythromycin (15 µg/disc), gentamicin (120 µg/disc), teicoplanin (30 µg/disc) and tetracycline (30 µg/disc) (HiMedia, India) were added on prepared inoculated plates with tested enterococcal strain, and then were incubated at 37 ± 1 °C. The sizes of zones were measured after 14 - 19 hours. The isolates were classified as susceptible, intermediate resistant or resistant.

RESULTS AND DISCUSSION

The counts of enterococci from teats reached the average value $2.77 \log_{10}$ cfu.ml⁻¹ and from teat cups of milking machine average value $2.85 \log_{10}$ cfu.ml⁻¹. The results of enterococci counts are shown in Table 1. Significant fluctuation of enterococci is probably related with differences in hygiene of udder and milking machines on farms. **Kagkli et al. (2007)** also confirmed the presence of enterococci in teat rinse with average value $1.50 \log_{10}$ cfu.ml⁻¹. This result shows that wrong sanitation of the udder and the milking machines is the major source of enterococci in raw milk. The cleanliness of cow can also affect the efficiency of cow preparation before milking, where dirty cows can double cow preparation time (**Reneau, Bey, 2007**). The cleanliness of the udder and teats can be influenced by several factors, including

transition from summer grazing to winter housing (housed cows being dirtier than grazing cows) (**Ellis et al., 2007**), faecal consistency (where increasingly fluid faecal consistency correlated with dirtier cows), frequency of bedding change and quality of bedding (**Ward et al., 2002**), and stage of lactation (**Reneau et al., 2005**). **Monsallier et al. (2012)** researched microbial flora on teat skins of 96 cows from 16 farms, which were sampled before washing and during milking. The counts of enterococci were at a level below $3 \log_{10}$ cfu.ml⁻¹. The authors noted that cleanliness of dairy cows reflects farming practices and influences the counts of microbial flora on teat skin. It offered prospects to better control teat microbial balance taking into account the milking hygiene practices, the parturition and the type of animal housing.

A total number of 99 enterococci isolates were collected. The results of species distribution are shown in Figures 1 and 2. The dominant species isolated from skin of teats (n=20) (Figure 1) and also from teat cups (n=18) (Figure 2) were representatives of *E. group III* - *E. durans*, *E. hirae*, *E. faecalis asaccharolytic var.* **Kagkli et al. (2007)** isolated *E. casseliflavus*, *L. mucosae*, *L. brevis*, *Aerococcus* sp. from the cow teats and in the case of some strains of enterococci, the origin from bovine faeces was confirmed. This is implying that cross-contamination from faeces to teats occurs. Other studies examined prevalence of enterococci isolated from faecal and environmental samples of dairy cattle. The highest percentage of enterococci strains were *E. hirae* 49.2%, *E. faecalis* 14.2%, *E. faecium* 13.4% and *E. casseliflavus* 10.4% isolated from faecal samples (**Jackson et al., 2010**). **Pradhan et al. (2009)** found the high prevalence of *Enterococcus* spp. (75 to 100%) isolated from environmental samples what indicate, they were ubiquitous throughout the farm environment.

Antibiotic resistance profiles of total enterococci isolated from skin of teats are shown in Figure 3. Isolates showed small resistance to two antibiotics. Of 49 isolates of enterococci, 4% of them were resistant to tetracycline and 4% of them were resistant to erythromycin. Intermediary resistance to tetracycline 8%, to erythromycin 25% and to vancomycin 8% was found. No resistance to the antibiotic teicoplanin, ampicillin and gentamicin was found. **Ma et al. (2006)** isolated *Enterococcus* spp. (n=271) from environmental (n=56), human (n=16) and dairy cows samples (n=199). Samples collected from dairy cows were

Table 1 Counts of enterococci (\log_{10} cfu.ml⁻¹) on skin of teat (n=58) and teat cups of milking machine (n=59)

	Skin of teat	Teat cups of milking machine
x_g	2.77	2.85
x_{min}	2	2
x_{max}	5.31	5.24
s_x	0.78	0.74

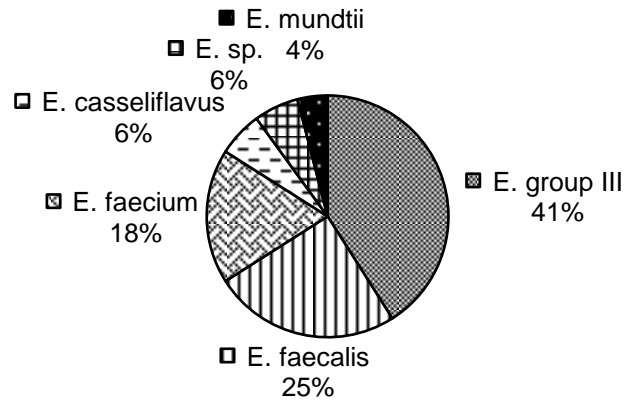


Figure 1 Distribution of species *Enterococcus* (n=49) isolated from skin of teats

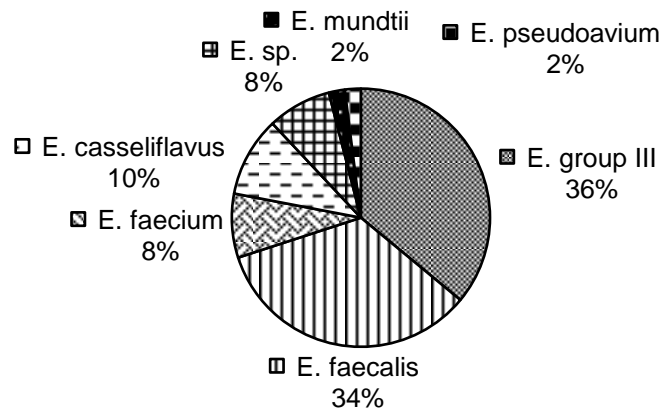


Figure 2 Distribution of species *Enterococcus* (n=50) isolated from teat cups of milking machine

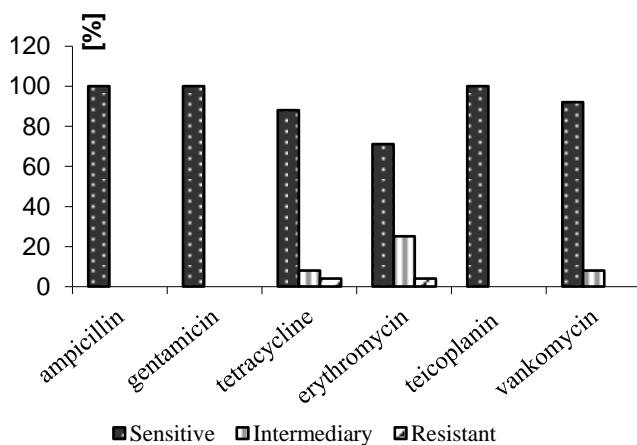


Figure 3 Antibiotic resistance profiles of enterococci isolated from skin of teats

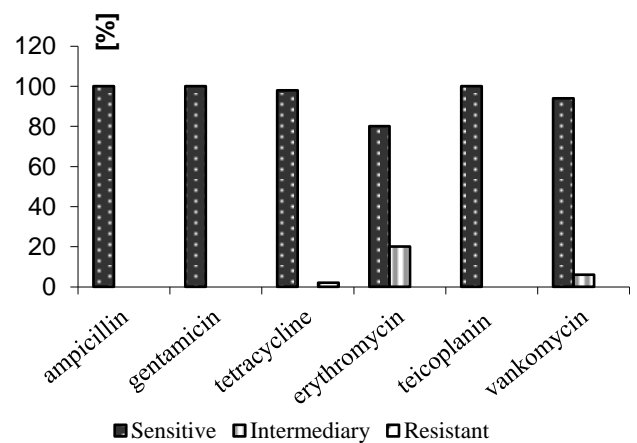


Figure 4 Antibiotic resistance profiles of enterococci isolated from teat cups

From the tested antibiotics, the highest levels of intermediary resistance had enterococci to tetracycline, erythromycin and vancomycin. One of the most widely used antibiotics combinations for dry cow treatment and prevention of mastitis is tetracycline and erythromycin.

In contrast, none resistant isolates to the antibiotic teicoplanin, ampicillin and gentamicin was found. In the case that the results of our research would be evaluated by older NCCLS requirements (e.g. from the year 1999), the proportion of resistant isolates would be higher.

Based on our or other authors' results, we do not support the unwarranted using of antibiotics in veterinary medicine because they are important driving force for selection of antibiotic resistant bacteria.

CONCLUSION

The results of this study show that there are high counts of enterococci in surface of cow udder and milking equipment. Besides fact that these enterococci can cause diseases of the mammary gland - mastitis, they may be also transmitted via milk and dairy products to the food chain of humans. Risk of enterococci for humans consist in the possible transfer of antibiotic resistance genes to other bacteria, including bacteria that are pathogenic to humans.

The relationship between the isolates of enterococci in the studied samples and tested antibiotics is not very clear and needs more investigation for better regulation strategies of safe using of antibiotics in farms.

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